Enterococci by Membrane Filtration
EPA 1600

Facility Name:_____VELAP ID_____

Assessor Name:_____Analyst Name:_____Inspection Date_____

Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
Records Examined: SOP Number/ Revision/ Date _____ Analyst:_____ Sample ID: _____ Date of Sample Preparation:_____ Date of Analysis:_____					
Are pipets sterile, glass or plastic, and of appropriate volume?	6.5				
Are graduated cylinders and membrane filter units sterilized and kept wrapped in foil or kraft paper?	6.0				
Are membrane filters, sterile, white grid marked, 47mm diameter, w/ 0.45 ± 0.02µm pore size used?	6.19				
Is the incubator maintained at 41 ± 0.5˚C?	6.21				
Is the waterbath maintained at 50˚C for tempering agar?	6.22				
Are the stock and working phosphate buffer solutions sterilized by filtration, or by autoclaving at 121˚C for 15 min and the stock then stored in the refrigerator?	7.5.2 7.5.5				
Is the mEI medium autoclaved at 121˚C for 15 min and cooled in a 50˚C water bath?	7.6.2				
Is the mEI agar poured into 9x50 mm petri plates to a depth of 4-5mm and allowed to solidify?	7.6.5				
Is the final pH of the medium 7.1 ± 0.2 and stored in the refrigerator?	7.6.5				
Are samples preserved at <10˚C and analyzed within 8 hours of collection? *"The maximum transport time to the laboratory is 6 hours, and samples should be processed within 2 hours of receipt at the laboratory."*	40 CFR 136.3 Table II				
Is the sample shaken vigorously at least 25 times?	11.4				
Are sample volumes chosen to produce 20-60 colonies? (Multiple volumes of the same sample or sample dilutions may be filtered and the results combined.)	11.5 11.6				
Notes/Comments:					

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After the sample is filtered, are the sides of the funnel rinsed at least twice with 20-30mL of sterile buffered rinse water?	11.7				
Is the filter aseptically removed and rolled onto the modified mEI agar to avoid the formation of bubbles?	11.8				
Is the dish inverted and incubated at 41 ± 0.5˚C for 24 hours?	11.8				
After incubation, are the plates counted using magnification and a small florescent lamp, and colonies with a blue halo recorded?	11.9				
Is enterococci count calculated as follows? Enterococci/100 mL = $\frac{\text{enterococci colonies counted}}{\text{mL sample filtered}}$ X100	12.1.1				
Are at least three volumes filtered per sample and reported as Enterococci per 100mL of sample? *(NOTE: This is recommended since method says "should".)*	14.1				
Is the optional verification test performed upon request?	15.1				
If prepared by the laboratory for the verification test, is the brain heart infusion broth (BHIB), BHIB with 6.5% NaCl and brain heart infusion agar (BHIA) autoclaved at 121˚C for 15min, with a final pH of 7.4 ± 0.2?	7.7.2 7.9.2				
If prepared by the laboratory for the verification test, Is the bile esculin agar (BEA) autoclaved at 121˚C for 15min, cooled in a 50˚C water bath with a final pH of 6.6 ± 0.2 and stored in the refrigerator?	7.10.2				
Are cells from the centers of at least 10 well-isolated typical colonies transferred to BHIB tubes and onto BHIA slants using a sterile inoculating needle?	15.2				

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Are broth tubes Incubated for 24 hr and slants incubated for 48 hr at 35 ± 0.5°C?	15.2				
After incubation, is a loopful of material from each BHIB tube transferred to BEA, BHIB, and BHIB with 6.5% NaCl?	15.3				
Is the BEA and the BHIB with 6.5% NaCl incubated at 35 ± 0.5°C for 48 h and observed for growth?	15.3.1 & 15.4				
Is the BHIB incubated at 45 ± 0.5°C for 48 h and observed for growth?	15.3.2 & 15.4				
After 48 h incubation, is a Gram stain applied to growth from each BHIA slant?	15.5				
Are Gram-positive cocci that grow and hydrolyze esculin on BEA (*i.e.*, produce a black or brown precipitate), and grow in BHIB at 45 ± 0.5°C and BHIB with 6.5% NaCl at 35 ± 0.5°C verified as enterococci?	15.6				

Notes/Comments: